Final AFOSR Progress Report Form (used for grants issued on or after 2/13/2006)

FINAL REPORT: February 2007 - April 2010

1. Cover Sheet:

- **Date:** May 10, 2010

- **Title**: Modulating Radiation Resistance: Novel Protection Paradigms Based on Defenses against Ionizing Radiation in the Extremophile *Deinococcus radiodurans*

- **Project 105257/AFOSR Award**: FA9550-07-1-0218. First Funded: April, 2007.

- **Present Status: End**

- **Technical Topic Area: 'Extremophile Initiative' - AFOSR Science Elements Addressed: 1) *Discover the mechanisms for survival in extremophiles*; and 2) *Explore methods for exporting these protective strategies outside of the host cell*.**

- **First Annual Report:** Submitted by M. J. Daly on December 10, 2007.
  Second Annual Report: Submitted by M. J. Daly on December 2, 2008.
  Third Annual Report: Submitted by M. J. Daly on December 9, 2009.

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- **Program Financial Management**: Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc. 1401 Rockville Pike, Suite 600, Rockville, MD 20852. HMJF provides USUHS support in research administration, program management and staffing, clinical trials, and education support.

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14. ABSTRACT

For Deinococcus radiodurans and other bacteria which are extremely resistant to ionizing radiation (IR) and desiccation, a mechanistic link exists between resistance, manganese accumulation, and protein protection. We have demonstrated that ultrafiltered, protein-free preparations of D. radiodurans cell extracts prevent protein oxidation at massive doses of IR. In comparison, ultrafiltrates from IR-sensitive bacteria were not protective. The D. radiodurans ultrafiltrate was enriched in Mn, phosphate, nucleosides, and bases and peptides. When combined in vitro at concentrations approximating those in D. radiodurans, these constituents interacted highly synergistically and formed complexes which preserved the activity of large, multimeric enzymes exposed to 50,000 Gy, conditions which obliterated DNA. When applied in vivo, they protected Escherichia coli and human cells from extreme cellular insults caused by IR. By establishing how proteins can be protected against indirect damage caused by gamma-rays delivered in vast doses, our findings provide the basis for a new approach to radioprotection.

15. SUBJECT TERMS
Novel manganese-dependent small-molecule radioprotectors; desiccation protectors; manganese peptide antioxidants; manganese-nucleoside-phosphate antioxidants; superoxide; enzyme protection; specific inhibitors of protein oxidation; preventing cellular damge caused by ionizing radiation and ultraviolet light. Deinococcus radiodurans; Lactobacillus plantarum; cyanobacteria; radiation resistant bacteria.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Michael Daly, PhD
U	U	U	UU	6	19b. TELEPHONE NUMBER *(Include area code)* (301)295-3750

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2. Objectives:

The original global objectives as listed in Daly's 2006 AFOSR application:

(1) Develop quantitative assays for oxidative protein damage as indicators for radiation and desiccation damage in prokaryotic and eukaryotic cells; and

(2) Develop novel radioprotectors and antioxidants based on catalytic Mn(II,III) redox-cycling processes identified in *D. radiodurans*.

Objective for Year-1: **Examine the mutual chemical nature of desiccation and radiation resistance in *D. radiodurans* using protein carbonylation immunoassays.**

Objective for Year-2: **Identify the chemical constituents of radioprotective antioxidant complexes of *D. radiodurans* using high performance liquid chromatography (HPLC) and mass spectrometry.**

Objective for Year-3: **Harness the antioxidant complexes of *D. radiodurans* for practical purposes for purified enzymes and living cells (*E. coli* and human Jurkat T cells).**

3. Summary of Effort (150 words):

For *Deinococcus radiodurans* and other bacteria which are extremely resistant to ionizing radiation (IR) and desiccation, a mechanistic link exists between resistance, manganese accumulation, and protein protection. We have demonstrated that ultrafiltered, protein-free preparations of *D. radiodurans* cell extracts prevent protein oxidation at massive doses of IR. In comparison, ultrafiltrates from IR-sensitive bacteria were not protective. The *D. radiodurans* ultrafiltrate was enriched in Mn, phosphate, nucleosides, and bases and peptides. When combined *in vitro* at concentrations approximating those in *D. radiodurans*, these constituents interacted highly synergistically and formed complexes which preserved the activity of large, multimeric enzymes exposed to 50,000 Gy, conditions which obliterated DNA. When applied *in vivo*, they protected *Escherichia coli* and human cells from extreme cellular insults caused by IR. By establishing how proteins can be protected against indirect damage caused by gamma-rays delivered in vast doses, our findings provide the basis for a new approach to radioprotection.

4. Progress for period March 2007 – April 2010:

In 2008, Dr. De Long advised Dr. Daly to continue to focus on characterizing the radioprotective effects of *D. radiodurans* Mn complexes on purified enzymes and cells. He advised to shift resources which were being used to look at desiccation resistance to radiation resistance. This was good advice as studies on desiccation resistance take months compared to those which study radiation effects, which take days to weeks.

Summary of objectives and results of Year-1: **Examine the mutual chemical nature of desiccation and radiation resistance in *D. radiodurans***. Extreme resistance to desiccation and radiation are mechanistically coupled in diverse organisms. However, the chemical basis of resistance to these oxidizing conditions in Mn-accumulating bacteria had not been investigated. We demonstrated that protein oxidation during desiccation is the most probable cause of cell death in desiccated bacteria, a finding which parallels those for irradiated bacteria. The program

manager at the time was Dr. Jennifer Gresham, who was interested in desiccation resistance mechanisms. When Dr. Hugh De Long took over the program he advised that Daly's group focus principally on ionizing radiation, which they did in Years 2 and 3.

Summary of objectives and results of Year-2: **Identify the chemical constituents of radioprotective antioxidant complexes of *D. radiodurans*.** Since 2004, Daly's group has been exploring the role of manganese accumulation in extremely radiation resistant bacteria. In Year-2, his group examined the nature of the chemical antioxidant defense mechanisms promoted by Mn, which specifically protect proteins from oxidative damage. This exploratory study was the first to examine radioprotective Mn complexes in any organism, and revolved around the bacterium *D. radiodurans*, which represents life's utmost limit for ionizing radiation resistance. The great efficiency of the seemingly ordinary DNA repair systems of *D. radiodurans* has been attributed to chemical antioxidant systems; the chemical antioxidants have now been characterized. The major antioxidant complexes of *D. radiodurans* consist of Mn, phosphate, peptides and nucleosides.

Summary of objectives and results of Year-3: **Harness the antioxidant complexes of *D. radiodurans* for practical purposes.** Since the 1960s, the goal of exporting the radioprotective processes of *D. radiodurans* outside of the host cell for practical purposes has eluded researchers. Daly's findings have moved such possibilities from the realm of speculation to plausible reality. In 2009, his group reconstituted the major antioxidant Mn complexes of *D. radiodurans* with astonishing levels of radioprotection. *In vitro*, they preserved the activity of enzymes exposed to immense doses of gamma-rays, but they did not significantly protect DNA. When applied *in vivo*, they protected bacteria and human cells from extreme cellular insults caused by gamma-irradiation.

Overview of Years 1-3: The bacterium *Deinococcus radiodurans* represents life's utmost limit for ionizing radiation (IR) resistance. In spite of intensive efforts, the goal of exporting the protective processes of *D. radiodurans* outside of the host cell for practical purposes has eluded researchers. In 1964, Alan K. Bruce published a paper (Radiat. Res. 22, 155) reporting a low molecular weight, protein-free extract from *D. radiodurans* which protected sensitive bacteria against the lethal effects of IR. However, the active components of the extract, and the cellular molecules they protected, were not identified. My group has been collaborating with Dr. Rodney L. Levine. He is Chief of the Laboratory of Biochemistry, NHLBI, NIH where his long-time colleague, the late Earl Stadtman, discovered and characterized an unexpected property of complexes consisting of Mn, amino acids and peptides, namely their ability to catalytically scavenge oxygen radicals. We have reconstituted the Mn-dependent chemical antioxidant systems of *D. radiodurans* with astonishing levels of radioprotection. In vitro, they preserved the activity of enzymes exposed to immense doses of IR. In vivo, they protected bacteria and human cells from extreme cellular insults caused by IR.

The antioxidant complexes of *D. radiodurans* protein-free extracts are comprised of Mn, phosphate, nucleosides and bases, and peptides. When combined in vitro at concentrations present in *D. radiodurans*, these constituents interacted synergistically and preserved the activity of large, multimeric enzymes exposed to 50,000 Gy, conditions which obliterated DNA. When these agents and their analogues were applied to the bacterium Escherichia coli, the cells displayed luxuriant growth under high-level chronic irradiation delivered at dose rates which kill all but the most IR-resistant bacteria. Collectively, our findings resolve how, after exposure to huge doses of gamma-rays, or to months of desiccation in a desert, *D. radiodurans* cells retain sufficient protein activity to reconstitute their DNA.

Our results hold theoretical and practical implications of the highest order. For example, antioxidant Mn complexes may be acting similarly in other organisms, and could explain the

independent evolution of extreme IR resistance in the three domains of life. Practical areas which could be impacted include bioremediation of high-level radioactive waste sites, and metabolic interventions at the cellular level which mitigate oxidative stress during irradiation or aging. Another tangible application of our approach is the preparation of IR-sterilized whole-bacterial cell, whole-virus, and protein vaccines without loss in immunogenicity. A recent mathematical model of radiogenic oxidative stress is consistent with our data, which can potentially be generalized to other organisms and lower radiation doses (I. Shuryak, D. J. Brenner, J. Theor. Biol. 261, 305 (2009)).

On August 10, 2009, I presented these results at the Gordon Research Conference on Cell Biology of Metals in Newport, RI. There was general agreement among participants that our findings represent a breakthrough with widespread implications in the field of oxidative stress management. This research was also presented at the John B. Little Symposium at Harvard's Center for Radiation Sciences on Oct. 23, 2009.

5. Personnel Supported: List professional personnel (Faculty, Post-Docs, Graduate Students, etc.) supported by and/or associated with the research effort.

Personnel: 1 PI and 2 Research Associates funded by AFOSR grant:

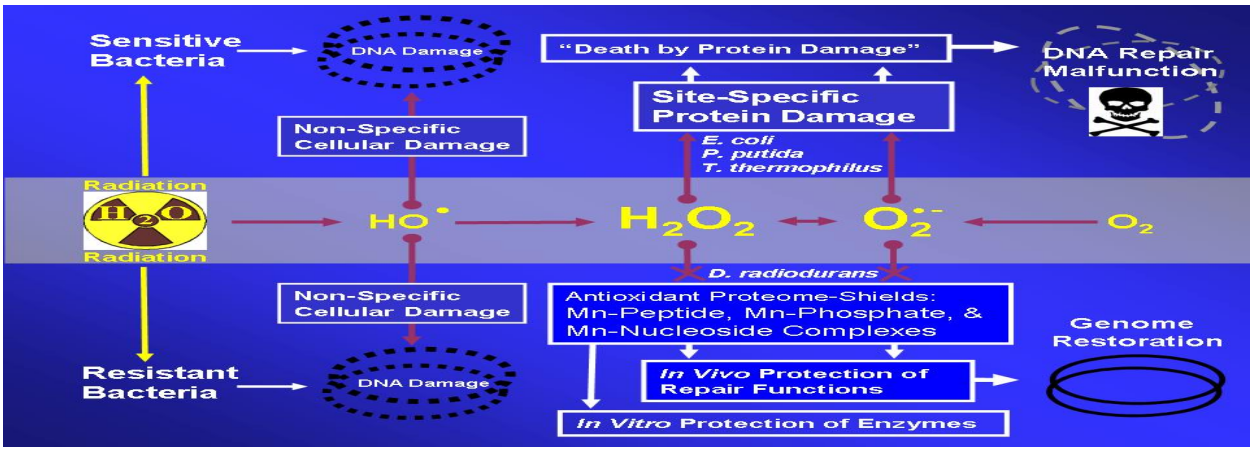
PI: M. J. Daly, Ph.D., Professor (no salary from AFOSR grant)
Senior Res. Associate: Dr. Elena Gaidamakova, Ph.D. (100% salary from AFOSR grant)
Senior Res. Associate: Dr. Vera Matrosova, Ph.D. (100% salary from AFOSR grant)

Principal Collaborator: No Funds: Rodney Levine, M.D., Ph.D., Chief, Laboratory of Biochemistry, NHLBI, NIH. Dr. Levine is the world's leading authority on protein carbonylation and has made resources including irradiators in his lab available to Daly's group.

6. Publications: List peer-reviewed publications and abstracts submitted and/or accepted during the 36-month period.

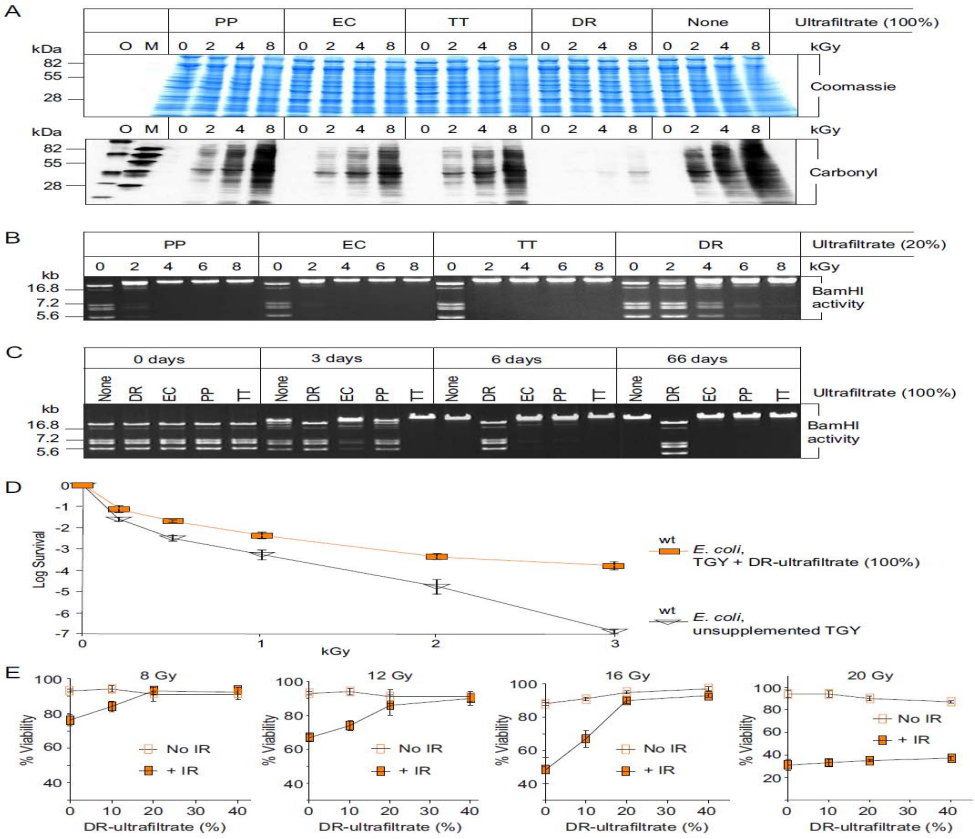
1. **MICHAEL J. DALY** (2010) Death by protein damage in irradiated cells. To be submitted.

Abstract | The modern founding concept of radiation biology that deals with x-rays and γ-rays is that ionizing radiation is dangerous because of its damaging effects on DNA. Mounting experimental evidence does not fit into this theoretical framework, instead supporting that radiation resistance is governed by protein damage. Whereas DNA lesion-yields in cells exposed to a given dose of radiation appear to be fixed, protein-lesion yields are variable and closely related to survival. A revised basic theory of radiation toxicity is presented which accommodates the disparity between *in vivo* radiation effects on DNA and proteins. Implied major challenges to generally accepted views in radiation toxicity are presented together with interdisciplinary significance and future directions.



2. **MICHAEL J. DALY**, E. K. Gaidamakova, V. Y. Matrosova, J. G. Kiang, R. Fukumoto, D.-Y. Lee, N. B. Wehr, G. A. Viteri, B. S. Berlett, and R. L. Levine (2010) Small-molecule antioxidant proteome-shields in *Deinococcus radiodurans*. To be submitted.

Abstract | For *Deinococcus radiodurans* and other bacteria which are extremely resistant to ionizing radiation (IR) and desiccation, a mechanistic link exists between resistance, manganese accumulation, and protein protection. We show that ultrafiltered, protein-free preparations of *D. radiodurans* cell extracts prevent protein oxidation at massive doses of IR. In contrast, ultrafiltrates from IR-sensitive bacteria were not protective. The *D. radiodurans* ultrafiltrate was enriched in Mn, phosphate, nucleosides and bases, and peptides. When reconstituted *in vitro* at concentrations approximating those in *D. radiodurans*, these constituents interacted synergistically and formed complexes which preserved the activity of large, multimeric enzymes exposed to 50,000 Gy, conditions which obliterated DNA. When applied *in vivo*, they protected *Escherichia coli* and human cells from extreme cellular insults caused by IR. By establishing how proteins can be protected against indirect damage caused by gamma-rays delivered in vast doses, our findings provide the basis for a new approach to radioprotection.



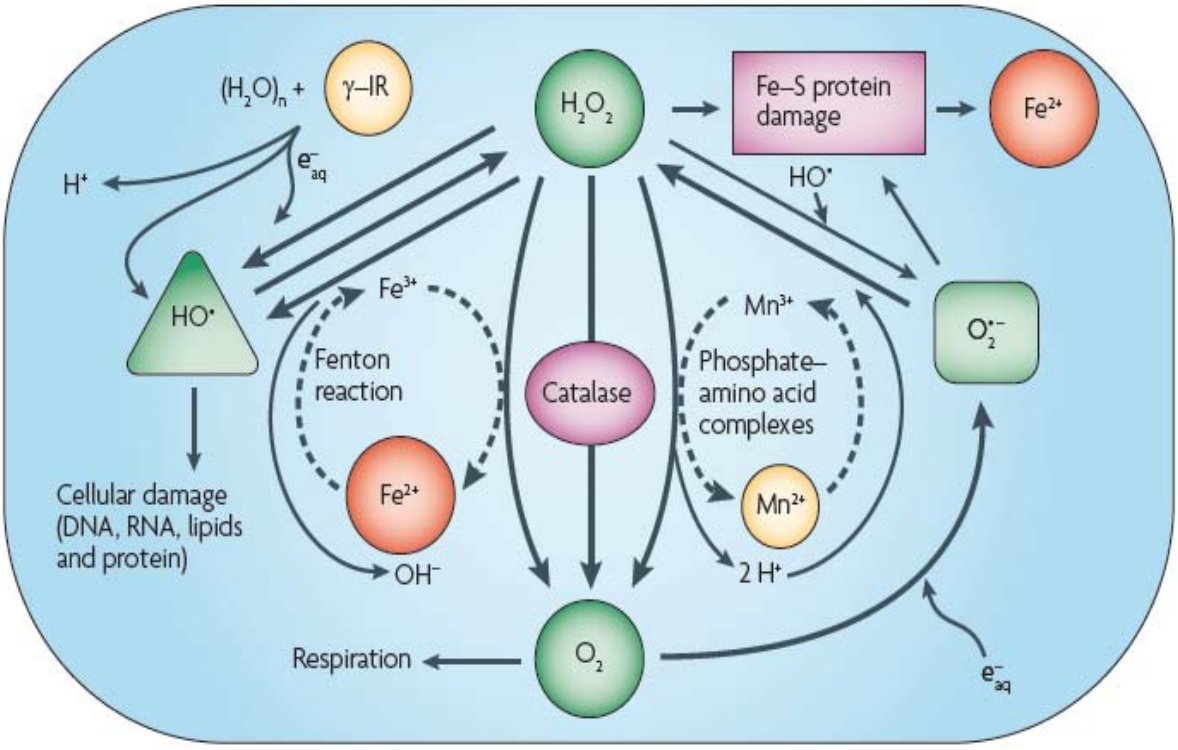
In vitro and *in vivo* protection by *D. radiodurans* (DR) ultrafiltrate. (**A**) DR-ultrafiltrate prevents protein oxidation. The indicated ultrafiltrates were mixed with purified *E. coli* proteins and irradiated to the indicated doses of γ-radiation (kGy). Proteins were then separated by polyacrylamide gel electrophoresis and visualized by Coomassie staining. Duplicate gels were subjected to Western blot carbonyl analysis, which reveals the presence (black) or absence (no signal) of protein oxidation. PP, *P. putida*; EC, *E. coli*; TT, *T. thermophilus*; and DR, *D. radiodurans*. O and M, size-standards. (**B**) DR-ultrafiltrate preserves the activity of an irradiated enzyme. *Bam*HI was irradiated in the indicated ultrafiltrates, then incubated with ⌊-DNA and subjected to agarose gel electrophoresis. (**C**) DR-ultrafiltrate preserves the activity of a desiccated enzyme. *Bam*HI was desiccated from the indicated ultrafiltrates and stored in a desiccator for the indicated times, and then assayed for residual activity as in panel B. (**D**) DR-ultrafiltrate protects *E. coli*. Wild-type *E. coli* (MM1925) cells were grown in TGY medium supplemented with DR-ultrafiltrate and irradiated without change of broth to the indicated doses, then recovered on TGY medium. Colony forming unit (CFU) survival assays were in triplicate for each dose, with standard deviations shown. (**E**) DR-ultrafiltrate protects human Jurkat T cells. DRultrafiltrate was added to the growth medium 1 day before irradiation.

3. K. S. Makarova and **MICHAEL J. DALY** (2010) Comparative genomics of stress response systems in *Deinococcus* bacteria. *Bacterial Stress Responses* (In Press)

Abstract | The prospect of comparative genomics resolving the seemingly paradoxical mechanism of extreme radiation resistance in *Deinococcaceae* is growing. Two additional whole-genome sequencing projects for *Deinococcus* are underway at the US Department of Energy's Joint Genome Institue; *Truepera radiovictrix* and *Deinococcus grandis* are expected to be completed in 2010. Based on historical and contemporary research, it now seems evident that the extreme IR resistance phenotype of *Deinococcaceae* stems from a subtle interplay between diverse, but widespread systems including Mn homeostasis, metabolite regulation, respiratory control, macromolecular degradation, and other oxidative stress response pathways.

4. **MICHAEL J. DALY** (2010) *Deinococcus radiodurans*: revising the molecular basis for radiation effects on cells. Extremophiles Handbook (In Press) Horikoshi K (Ed.).

Abstract | The field of radiobiology was built on the premise that radiation is dangerous because of its damaging effects on DNA, where only a few events, or even a single event, at the molecular level can inactivate cells. The discordance of modern radiation toxicity models with results spanning nearly five decades of research on the extremely radiation resistant bacterium *Deinococcus radiodurans* is reviewed. Much of the early data implicating DNA itself were for bacterial systems. However, recent studies show that extreme resistance to gamma-radiation among bacteria consistently coincides with a greatly diminished susceptibility to protein oxidation, but with similar DNA lesion yields as other organisms. A growing body of experimental evidence now supports that naturally sensitive bacteria are killed by radiation mainly owing to protein oxidation, whereas extreme resistance in bacteria is achieved by protecting enzymes and the repair functions they catalyze. Based on new insights, the prospects for exporting the radioprotective mechanisms outside of *D. radiodurans* for practical purposes are considered.



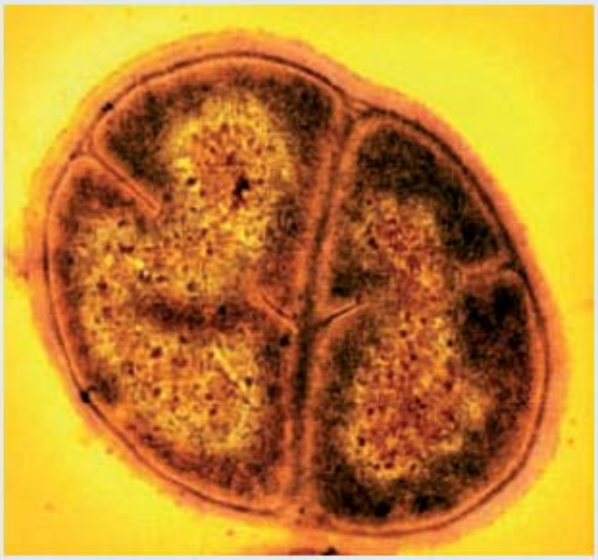
5. **MICHAEL J. DALY** (2009) *Deinococcus* prospects. *Nature Reviews Microbiology* **7**, 476.

Abstract | There is a growing realization that *D. radiodurans* did not evolve new antioxidant or DNA repair proteins, but rather that it found a way to protect conventional enzymes and use them more efficiently. Since the 1960s, the goal of exporting those protective processes outside of the host cell for practical purposes has eluded researchers. The day on which this goal will be achieved is not far off.

6. **MICHAEL J. DALY** (2009) A new perspective on radiation resistance based on *Deinococcus radiodurans. Nature Reviews Microbiology* **7**, 237.

Abstract | In classical models of radiation toxicity, DNA is the molecule that is mostvaffected by ionizing radiation (IR). However, recent data show that the amount of protein damage caused during irradiation of bacteria is better related to survival than to DNA damage. In this Opinion article, a new model is presented in which proteins are the most important target in the hierarchy of macromolecules affected by IR. A first line of defence against IR in extremely radiation-resistant bacteria might be the accumulation of manganese complexes, which can prevent the production of iron-dependent reactive oxygen species. This would allow an irradiated cell to protect sufficient enzymatic activity needed to repair DNA and survive.

Box 2 | **Where *Deinococcus* bacteria roam**



Representatives of the extremely radiation-resistant family Deinococcaceae can typically survive acute exposures to ionizing radiation (≥12,000 Gy (gray; absorbed radiation dose)), ultraviolet (≥1,000 J per m^2), and desiccation (years)[14,23,26,32,70], and can grow under harsh conditions of chronic irradiation (50 Gy per hour)[16]. Only approximately 30 distinct species have been described, despite their apparent ancient derivation[71]. The first member to be isolated was *Deinococcus radiodurans* (see the figure), originally named *Micrococcus radiodurans*[72]. This bacterium belongs to the *Deinococcus–Thermus* group, which is putatively related to cyanobacteria and is deeply branched in bacterial phylogenetic trees[23]. To date, the natural distribution of the deinococci has still not been explored systematically. Members have been isolated worldwide but have diverse and patchy distributions[23].

A wide range of natural and man-made environments on the Earth are characterized by their physical extremes of temperature, pressure and radiation. These harsh environments are different from the preferred environments of life typically encountered by humans, but can be colonized by *Deinococcus*. Some species live in highly radioactive soils at nuclear waste sites[73] and alpine environments[74], some have settled on sandstone, marble and ice in Antarctica[75], and others are ubiquitous microbial inhabitants of deserts[32]. High temperatures and pressures also do not seem to be an obstacle to their survival. *Deinococcus geothermalis* was originally isolated from a hot spring in Italy[76], and *D. geothermalis* DNA has been extracted from deep-ocean subsurface environments (68–118 meters below the sea floor)[77].

7. J. K. Fredrickson, S. W. Li, E. K. Gaidamakova, V. Y. Matrosova, M. Zhai, H. M. Sulloway, J. C. Scholten, M. G. Brown, D. L. Balkwill and **MICHAEL J. DALY** (2008) Protein oxidation: key to bacterial desiccation resistance? *ISME J*. **2**, 393.

Abstract | For extremely ionizing radiation-resistant bacteria, survival has been attributed to protection of proteins from oxidative damage during irradiation, with the result that repair systems survive and function with far greater efficiency during recovery than in sensitive bacteria. Here we examined the relationship between survival of dry-climate soil bacteria and the level of cellular protein oxidation induced by desiccation. Bacteria were isolated from surface soils of the shrub-steppe of the US Department of Energy's Hanford Site in Washington State. A total of 63 isolates were used for phylogenetic analysis. The majority of isolates were closely related to members of the genus *Deinococcus*, with *Chelatococcus*, *Methylobacterium* and *Bosea* also among the genera identified. Desiccation-resistant isolates accumulated high intracellular manganese and low iron concentrations compared to sensitive bacteria. In vivo, proteins of desiccation-resistant bacteria were protected from oxidative modifications that introduce carbonyl groups in sensitive bacteria during drying. We present the case that survival of bacteria that inhabit dry-climate soils are highly dependent on mechanisms, which limit protein oxidation during dehydration.

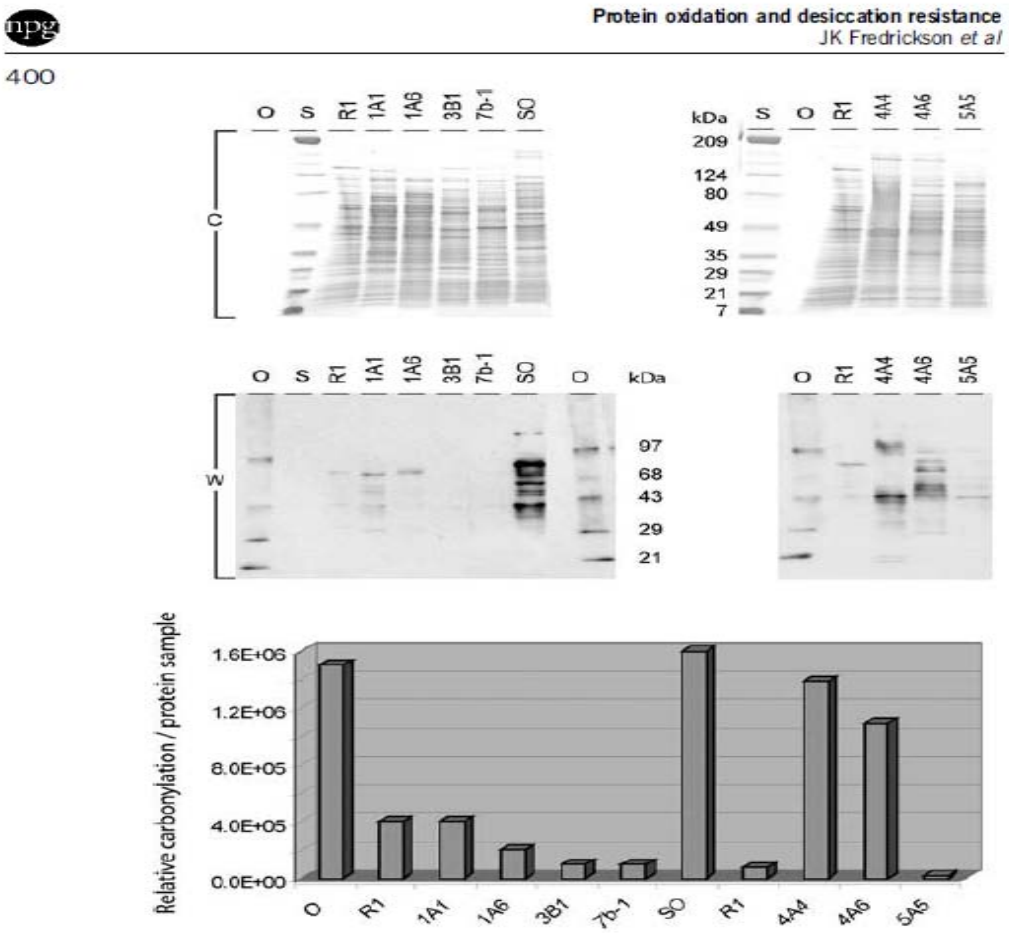


Figure 4 *In vivo* desiccation-induced oxidative protein damage. C, coomassie-stained polyacrylamide denaturing gel of 5 μg total soluble protein per lane for the indicated strains. W, western blot immunoassay of protein-bound carbonyl groups introduced into the proteins (5 μg per lane) by oxidative reactions following desiccation of the bacteria for 6 days. O, oxidized protein standards. S, wide-range protein size standards. R1, *Deinococcus radiodurans* R1 and SO, *Shewanella oneidensis* MR-1. Bottom, densitometric quantification of total protein carbonyl levels per lane (W).

8. B. Lai, S. Vogt, J. Mase, B. Ravel, K. Kemner and **MICHAEL J. DALY** (2007) Applications and future prospects for x-ray fluorescence microprobe analysis. Microscopy and Microanalysis, **13**, 1426.

Abstract | X-ray fluorescence microscopy is ideally suited for trace metal profiling and quantification due to its inherent elemental sensitivity of ~ 0.1-10 parts per million (ppm). A finely focused x-ray beam of 5- 30 keV is used to excite characteristic x-ray emissions from a specimen, and the total metal concentration can be measured directly without any labeling with fluorescent sensors. Currently, a spatial resolution of ~ 200 nm is achieved routinely at the 2-ID-D station of the APS, with a minimum detection limit as low as 3 attograms for zinc (2.7 x 10^4 atoms) within one second of data acquisition time.

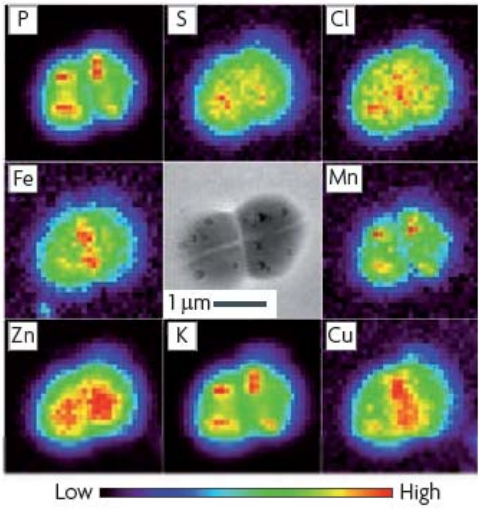


Figure 4 | **X-ray fluorescence maps of the qualitative distribution and concentration gradients of various metals and non-metals in *Deinococcus radiodurans*.** The tetracoccus was harvested from a mid-logarithmic culture in undefined rich medium, imaged and quantified as described previously[26,34]. The central panel is a transmission electron micrograph (TEM) of the tetracoccus after X-ray fluorescence (XRF) imaging. The panels that surround the TEM are the element distribution images of indicated metals and non-metals, which qualitatively depict high and low X-ray fluorescent intensities as indicated by the colour key (bottom): red represents the highest element concentration and black represents the lowest element concentration. A mathematical model of the original morphology of the cells was constructed in approximate likeness to the tetracoccus to determine the distribution of the elements[34]. XRF analysis measurements were made using the hard X-ray microprobe beamline 2ID-D at the Advanced Photon Source, Argonne National Laboratory, Chicago, USA, under previously described conditions[34,62]. Figure modified

9. **MICHAEL J. DALY**, E. K. Gaidamakova, V. Y. Matrosova, A. Vasilenko, M. Zhai, B. Ravel, B. Lai, R. D. Leapman, S.-M. W. Li, K. M. Kemner and J. K. Fredrickson (2007) Protein oxidation implicated as the primary determinant of bacterial radioresistance. *PLoS Biology* **5**(4) e92.

Abstract | In the hierarchy of cellular targets damaged by ionizing radiation (IR), classical models of radiation toxicity place DNA at the top. Yet, many prokaryotes are killed by doses of IR that cause little DNA damage. Here we have probed the nature of Mn-facilitated IR resistance in D*einococcus radiodurans*, which together with other extremely IR-resistant bacteria have high intracellular Mn/Fe concentration ratios compared to IR-sensitive bacteria. For in vitro and in vivo irradiation, we demonstrate a mechanistic link between Mn(II) ions and protection of proteins from oxidative modifications that introduce carbonyl groups. Conditions that inhibited Mn accumulation or Mn redox cycling rendered *D. radiodurans* radiation sensitive and highly susceptible to protein oxidation. X-ray fluorescence microprobe analysis showed that Mn is globally distributed in *D. radiodurans*, but Fe is sequestered in a region between dividing cells. For a group of phylogenetically diverse IR-resistant and IR-sensitive wild-type bacteria, our findings support the idea that the degree of resistance is determined by the level of oxidative protein damage caused during irradiation. We present the case that protein, rather than DNA, is the principal target of the biological action of IR in sensitive bacteria, and extreme resistance in Mn-accumulating bacteria is based on protein protection.

7. Most Recent Interactions/Transitions:

August 10-15, 2009, Daly presented his most recent results at the Gordon Research Conference on Cell Biology of Metals in Newport, RI. There was general agreement among participants that our findings represent a breakthrough with widespread implications in the field of oxidative stress management.

As the opening speaker, Daly presented his research at the John B. Little Symposium at Harvard's Center for Radiation Sciences on Oct. 23-24, 2009.

In 2010, as Invited Speaker: Society for General Microbiology (http://www.sgm.ac.uk/). In September 2010 (6th– 9th September), the SGM is holding its Autumn meeting at the Nottingham University Conference Centre, Nottingham, UK).

8. **Patent Disclosures**. Pending.

COMPOSITIONS CONTAINING PURINE AND PYRIMIDINE NUCLEOSIDES, PEPTIDES, PHOSPHATE AND MANGANESE AND THEIR USES.

FOR DETAILS: La Shaun J. Berrien, Ph.D., Director of Intellectual Property, Director, Joint (USU & HJF) Office of Technology Transfer, The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., 1401 Rockville Pike, Suite 600 Rockville, MD 20852

9. Honors/Awards: 'List honors and awards received during the grant/contract period.' NONE.